

Supplementary Information

Ester Bonds for Modulation of the Mechanical Properties of Protein Hydrogels

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Fgβ-(GB1-ParV)₂-cys:

MKLGSNEEGFFSARGHRPLDGS GSGSGSAGTGSGRSMDTYKLI L NGKTLK
GETTTEAVDAATAAEKVFKQYANDNGVDGEW TYDDATKTFTVTERS **GEIKT**
TVMAGGKTSTENEVATLKAKDIEGGVEVSDKIT YKGLYPNEKYDVIGKIY
EVKDGELVNP GHPVSVVNSGDDLKADTTGKGWTLNFGKLNLEAGKSYVV
FETVVS MENVIDTDGDGKPKKKQELTHEDPNDKSQTFRIRSMDTYKLI L N
GKTLKGETTTEAVDAATAAEKVFKQYANDNGVDGEW TYDDATKTFTVTERS
GEIKT**TVMAGGKTSTENEVATLKAKDIEGGVEVSDKIT YKGLYPNEKYDV**
IGKIYEVKDGELVNP GHPVSVVNSGDDLKADTTGKGWTLNFGKLNLEAG
KSYVVFETVVS MENVIDTDGDGKPKKKQELTHEDPNDKSQTFRIRSCGTE
FAAALEHHHHHH

SdrG-cys:

MKLGSEQGSNVNHLIKVTDQSIT EGYDDSDGI IKAHDAENLIYDVTFEVDD
KVKSGDTMTVNIDKNTVPSDLTDSFAIPKIKDNSGEI IATGTYDNTNKQIT
YTFTDYVDKYENIKAHLKLTSYIDKSKVPNNNTKLDVEYKTALSSVNKTIT
VEYQKPNENRTANLQSMFTNIDTKNHTVEQTIYINPLRYSAKETNVNISGN
GDEGSTIIDDSTI IKVYKVGDNQNLPSNRIYDYSEYEDVTNDDYAQLGNN
NDVNINFGNIDSPYI IKVISKYDPNKDDYTTIQQTVMQTTINEYTGERT
ASYDNTIAFSTSSGQGQGDLPPEKTRS CGTEFAAALEHHHHHH

cys-ParV₂-cys:

MRGSHHHHHH **CGSGEIKT****TVMAGGKTSTENEVATLKAKDIEGGVEVSDKIT**
YKGLYPNEKYDVIGKIYEVKDGELVNP GHPVSVVNSGDDLKADTTGKGWTL
NFGKLNLEAGKSYVVFETVVS MENVIDTDGDGKPKKKQELTHEDPNDKSQT
FRIRSGEIKT**TVMAGGKTSTENEVATLKAKDIEGGVEVSDKIT YKGLYPN**
EKYDVIGKIYEVKDGELVNP GHPVSVVNSGDDLKADTTGKGWTLNFGKLN
LEAGKSYVVFETVVS MENVIDTDGDGKPKKKQELTHEDPNDKSQTFRIRSG
TC

cys-GB1₂-cys:

MRGSHHHHHH **CGSMDTYKLI L NGKTLKGETTTEAVDAATAAEKVFKQYANDN**
GVDGEW TYDDATKTFTVTEGPQGIWGQMDTYKLI L NGKTLKGETTTEAVDA
ATAAEKVFKQYANDNGVDGEW TYDDATKTFTVTERSGTCT

Figure S1. Sequences for proteins Fgβ-(GB1-ParV)₂-cys, SdrG-cys, cys-ParV₂-cys and cys-GB1₂-cys. The sequence of ParV is shown in green and the ester bond within ParV is formed between T and Q residues.

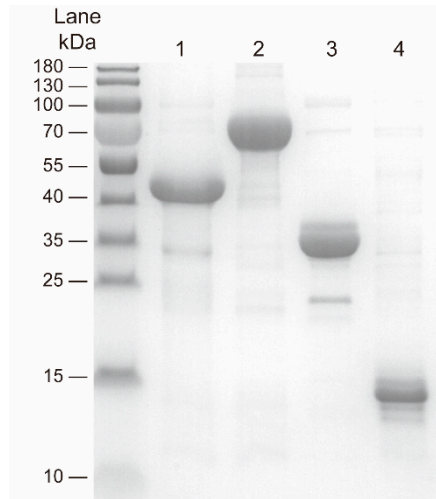


Figure S2. Proteins characterization by SDS-PAGE. Lanes 1-4 are SdrG-cys, Fg β -(GB1-ParV)₂-cys, cys-ParV₂-cys, cys-GB1₂-cys, respectively.

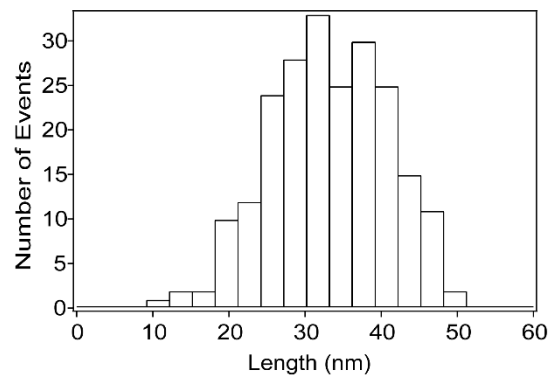


Figure S3. The histogram of the contour length before GB1 unfolding in Tris buffer at pH 7.4.

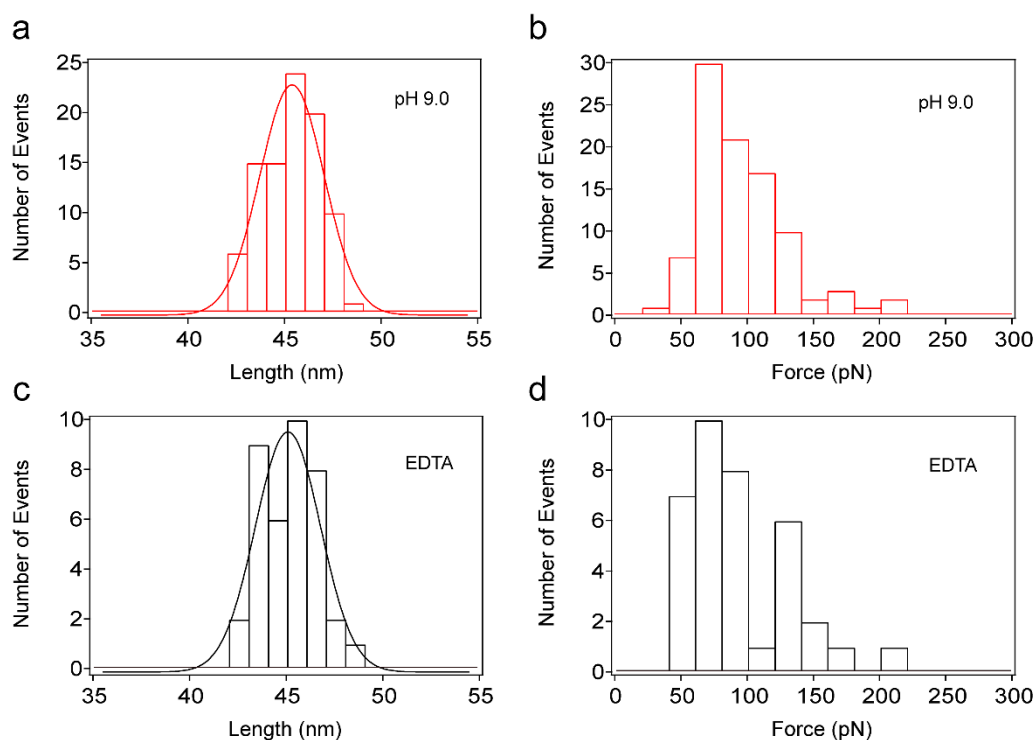


Figure S4. (a) Histogram of contour length increments (45.8 ± 2.32 nm) for the ester bond rupture events peaks at pH 9.0; (b) rupture force histogram of ester bond at pH 9.0; (c) histogram of contour length increments (45.4 ± 2.38 nm) for the ester bond rupture events peaks in Tris buffer with EDTA. (d) rupture force histogram of ester bond in Tris buffer with EDTA.

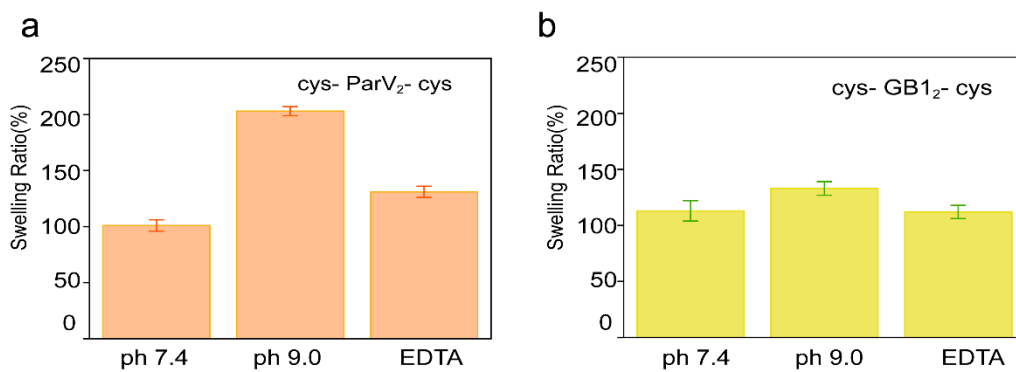


Figure S5. (a) Swelling measurement of cys-ParV₂-cys hydrogels under different conditions. The swelling ratios of the hydrogels at pH 7.4, at pH 9.0 and with 10 mM EDTA are as follows: 101% ± 5%, 203% ± 4% and 131% ± 5%, respectively; **(b)** Swelling measurement of cys-GB1₂-cys hydrogels under different conditions. The swelling ratios of the hydrogels at pH 7.4, at pH 9.0 and with 10 mM EDTA are as follows: 113% ± 9%, 133% ± 6% and 112% ± 6%, respectively. The hydrogels were taken out and weighed immediately after being removed from the mold, and the weight was recorded as W_0 . After swelling sufficiently and being immersed in different solutions, the hydrogels were removed and weighed again, with the weight recorded as W_i . The swelling ratio was calculated using following formula: Swelling ratio (%) = $[(W_i - W_0)/W_0] \times 100\%$.

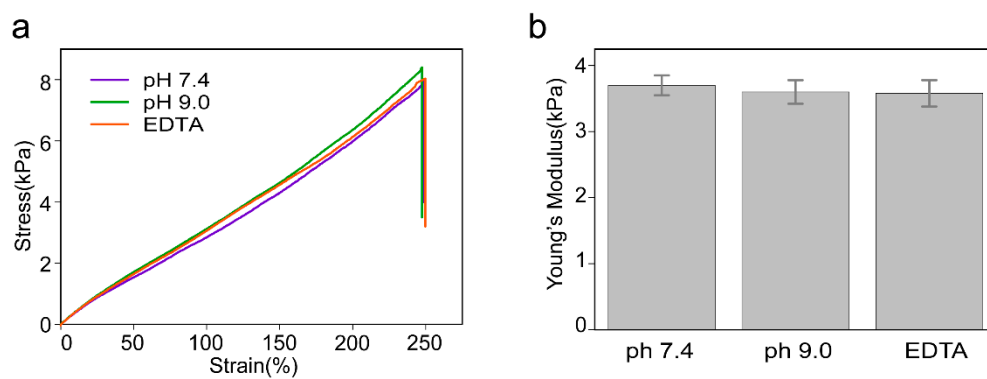


Figure S6. Tensile test experiments of cys-GB12-cys hydrogels. **(a)** Stress–strain curves for cys-GB12-cys hydrogels under different conditions; **(b)** Young's modulus of cys-GB12-cys hydrogels in three conditions.

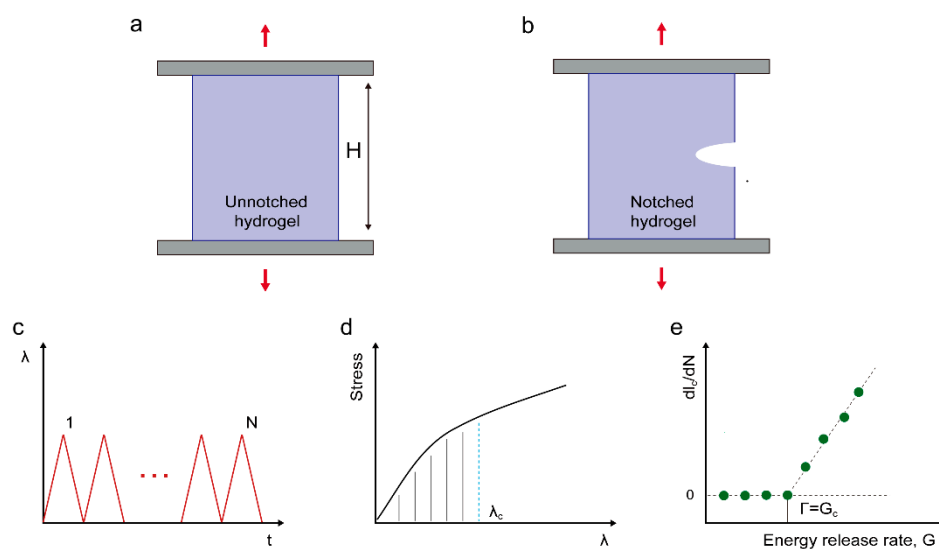


Figure S7. (a) Schematic of stretching unnotched hydrogel; (b) schematic of stretching notched hydrogel; (c) cyclic load/unload test; (d) stretch-strength curves; (e) illustration of measuring crack extension per cycle dl_c/dN versus energy release rate G curves.